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Quarterly Report July - Sept. 1989
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I. Work Summary

The first quarter has been devoted in part to organization, ordering, and reagent preparation. A significant change is that I have moved my laboratory which had been physically located in room 426 of the Gray building of the MGH to occupy a newer larger laboratory physically located in the new MGH research building, 10 minutes away in Charlestown. The move is an expansion of prior facilities, and is a clear cut improvement over the prior laboratory. The new laboratory is approximately 600 square feet, and is located together with five other members of the Infectious Disease faculty. The disadvantage of being a short shuttle bus away is already outweighed by the improved facilites. A description of the new performance site is appended on a separate sheet.

We have ordered, obtained, and are in the process of setting up the computer work station, the beta scintillation counter and the low pressure chromatography system.

As part of the work plan we have made killed whole cell vaccines to E. coli 0111B4 and S. typhimurium, and generated polyclonal rabbit antisera to each that have titers of 1:300,000 by ELISA. We are now purifying the IgG on DEAE sephacel anion exchange columns, and we are this week coupling S. typhimurium LPS to sepharose beads in order to affinity-purify the IgG. If the procedure goes without difficulty, we will couple the E. coli 0111B4 LPS in the next several weeks also.

We have ordered rabbits for the preparation of large pools of normal and tolerant sera to use as constant beginning material for the purification. We have also started to set up the cytotoxicity assay for tumor necrosis factor using 929 cells, a task made easier by a modern cell culture facility in the new laboratory. Our early results suggest that a murine macrophage cell line (RAW 264.7) will serve as a macrophage source (alleviating the cumbersome need for rabbit peritoneal cells), and our preliminary results with these cells indicate that the assay is sensitive to picograms/ml E. coli 0111B4 LPS. We will try this assay, in addition to limulus lysate, as an on line check for the neutralizing ability of our preparations during the purification.

II. New knowledge

In collaboration with Jean-Marc Cavaillon at Institute Pasteur in Paris, we have found that LPS complexed to lipoprotein (LPS-LP) is 20 to 1000-fold less active in stimulating murine and human

macrophages/monocytes to release IL-1, IL-6, and TNF. Binding experiments using radiolabeled reagents suggest that the decrease in cytokine activation and release that occurs results from an altered (and decreased) ability of LPS-LP to interact with the macrophage membrane. These results are being prepared for submission, and are relevant to the project in that the predominant endotoxin-induced neutralizing serum factor (which we are hoping to isolate) is found in the lipoprotein density range.

III. Technical problems - none

IV. Goals for next quarter

a. preparation of radiolabeled LPS (E. coli 0111B4, S. typhimurium)
b. preparation of large pools of normal and tolerant rabbit sera
c. baseline evaluation of pools for use as a starting material for purification
   1. ability of normal and tolerant pools to neutralize LPS in limulus and TNF assays
   2. formation of complexes of radiolabeled LPS in normal and tolerant pools as assessed by precipitation with calcium and dextran
d. affinity purification of anti-O polysaccharide IgG, and then preparation of anti-LPS immunoaffinity columns using this IgG
e. set up and calibration of molecular sizing columns

Description of Infectious Disease laboratory space of H. Shaw Warren, M.D.
5th Floor, MGH East, 149 13th Street, Charlestown, MA.

The laboratory consists of approximately 600 square feet amidst the larger Infectious Disease laboratory unit space, and an approximately 150 square foot office for the P.I. The space is divided into 2 modules with 4 benches and 2 desks. Available equipment include a refrigerator, low and high speed centrifuges, an ultracentrifuge, pH meter, pan and Mettler balances, -20°C and -70°C freezers with available space, liquid nitrogen tank, a Millipore water purification system distributing pyrogen-free water (less than 1 pg/ml LPS), dissecting and light microscopes, dark room, beta and gamma scintillation counters, a lyophilizer, a spectrophotometer, gel electrophoresis equipment, and an automated ELISA reader. There is in addition a warm room, cold room, walk-in -20°C freezer, and a tissue culture room with hood and CO_2 incubator for the sole use of the P.I. A refrigerated chromatography cabinet is on order for the use of pyrogen-free columns.